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Abnormally Large von Willebrand Factor Multimers in Henoch-Schönlein Purpura

Alessandra Casonato, Elena Pontara, Antonella Bertomoro, Elena Ossi, Massimo Vincenti, Antonio Girolami, Arturo Borsatti, and Giselda Bertaglia

Institute of Internal Medicine, Division of Nephrology (E.O., M.V., A.Bo., G.B.), Institute of Medical Semeiotic (A.C., E.P., A.Be., A.G.), Fourth Chair of Internal Medicine, University of Padua, Padua, Italy

Allergic vasculitis phenomena seem to be involved in Henoch-Schönlein purpura (HSP). Elevated plasma levels of von Willebrand factor (vWf) are a well recognized feature of vasculitis and have been taken as an indication of in vivo endothelial cell damage. Plasma factor VIII:C and vWf levels and vWf multimeric pattern were studied in 8 patients with HSP, during active disease and twice during the remission (3 and 9 months later). Plasma vWf multimeric composition was evaluated using low resolution gels which better resolve large vWf multimers. During active disease plasma factor VIII:C, vWf:Ag, and vWf:RCoF were normal in 5% of patients and increased in three, but in each patient, platelets appeared to aggregate at doses of ristocetin lower than in normals. Furthermore, all patients demonstrated the presence of abnormally large vWf multimers usually found only in platelets and endothelial cells. Three and 9 months later, during remission, in spite of the normalization of factor VIII:C and vWf levels, the abnormal multimers were still detectable, as well as hyper-responsiveness to ristocetin. These findings confirm that damage and/or perturbation of endothelial cells is associated with HSP. Moreover, the persistence of abnormality in the vWf multimeric pattern, when the disease is inactive, suggests that the mechanisms involved operate through the entire clinical course.

Key words: von Willebrand factor, Henoch-Schönlein purpura, endothelial cell damage

INTRODUCTION

Henoch-Schönlein purpura (HSP) is a clinical syndrome characterized by the association of non-thrombocytopenic purpura, arthralgias, abdominal pain, and glomerulonephritis [1]. The etiology of the disorder is unknown, but all clinical manifestations seem to be subordinate to an "allergic vasculitis" [2].

Von Willebrand factor (vWf) is a plasma glycoprotein that has a major role in supporting platelet adhesion to subendothelium during haemostasis [3]. vWf is synthesized and stored in megakaryocytes and endothelial cells, from which is secreted into the plasma [4] where vWf is organized into a set of multimers ranging from 450×10^3 to more than 20×10^6 daltons [5]. Cellular vWf shows a subset of high molecular weight multimers that are larger than those normally present in plasma [6,7] which are more active in causing platelet aggregation [8]. These unusually large multimers appear transiently in the plasma of normal subjects after the infusion of DDAVP [9,10] because this drug causes their release from their storage site in Weibel-Palade bodies. Since in another purpuric but thrombocytopenic disease like thrombotic thrombocytopenic purpura, an abnormality in plasma vWf multimeric composition had been reported [11–13] and considering that we found a similar behaviour of vWf in patients affected by IgA nephropathy [14], we decided to search for some derangement in vWf also in HSP. In this study, we demonstrate that plasma from HSP patients contains a subset of supranormal vWf multimers similar to those found within the endothelial cells. This is maintained, albeit to a lesser extent, throughout the entire clinical course of the disease.

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Address reprint requests to Giselda Bertaglia, M.D., Divisione di Nefrologia, Policlinico Universitario, Via Giustiniani, 2, 35125 Padua, Italy.

TABLE I. Main Clinical and Laboratory Findings for Eight Patients With Henoch-Schönlein Purpura*

Patients (sex/age, yr[a])	Disease duration	Purpuric episodes (n)	Arthralgias	Abdominal pain	Renal involvement: Hematuria (g/day)	Renal involvement: Proteinuria	Light/immunohistologic features
1 (M/18)	2 yr	3	No	Yes	Macro	1.4	IgA mesangial deposits
2 (M/19)	9 yr	Continuous relapsing	No	Yes	No	No	NIVD[b] (skin biopsy)
3 (M/16)	1 mo	2	No	Yes	Micro	9.7	IgA mesangial deposits
4 (M/14)	1 yr	5	Yes	Yes	Macro	3.5	IgA mesangial deposits
5 (F/29)	1 mo	1	No	Yes	No	No	Biopsy not performed
6 (M/23)	6 yr	5	No	No	Micro	No	[b]NIVD (skin biopsy)
7 (M/15)	6 yr	8	No	Yes	Micro	No	Biopsy not performed
8 (F/6)	7 mo	6	No	Yes	Macro	No	Biopsy not performed

*1990 criteria for the classification of Henoch-Schönlein purpura according to American College of Rheumatology [2].
[a]Present age.
[b]NIVD: neutrophilic inflammatory vascular disease [16].

MATERIALS AND METHODS

Patients

We studied five males and three females, ranging in age from 6 to 29 years (mean, 17.5 ± 6.7). These patients were selected according to the American College of Rheumatology (ACR) criteria for the classification of vasculitis [2] that include: age ≤20 years at disease onset, palpable purpura, acute abdominal pain, and biopsy showing granulocytes in the walls of small arterioles or venules. The presence of one or more of these criteria distinguishes HSP from other forms of vasculitis [15].

One patient (no. 2) fulfilled four out of ACR diagnostic criteria for HSP, while six patients (nos. 1,3,4,6,7,8) satisfied three out of four; patient no. 5 presented the minimum number of ACR criteria required for diagnosis (Table I) [16]. An acute infection was documented in patients nos. 1,4,7, and 8 prior to the development of purpura. Increased IgE levels were found in two patients. Patient no. 1 had a 1-week history of bronchial pneumonia at admission, and subsequently developed an acute renal failure that required dialysis. All patients, but one, underwent short-term, low-dose steroid treatment. All of the patients were evaluated during active disease and reexamined at least twice during the recovery period.

METHODS

Blood samples were obtained from patients and healthy volunteers following their informed consent, and in accordance with the declaration of Helsinki. Blood was collected into 3.8% sodium citrate as anticoagulant (1:9, v/v). Samples containing inhibitors of calcium-activated proteases were anticoagulated with 50 mM EDTA, 50 IU/ml trasylol and 3.85% sodium citrate. Platelet rich plasma (PRP) was prepared by centrifuging blood samples at 180*g* for 10 min.

Platelet poor plasma (PPP) was obtained by centrifuging blood samples at 1,000*g* for 15 min. Ristocetin-induced platelet aggregation was performed in a siliconized glass cuvette at 37°C with continuous stirring at 1,200 RPM in a chrono-log lumiaggregometer (Chrono-log, Havertown, PA).

vWF antigen (vWf:Ag), vWf ristocetin-cofactor activity (vWf:RCoF), and factor VIII:C were assayed, as previously described [17,18].

Platelet vWf:Ag contents were determined by a previously described ELISA technique [18]. Briefly, platelets from EDTA-collected samples were washed three times in PBS, and then lysed by adding 1% Triton X-100. Before lysing, the platelet number was adjusted to 10^6/μl (final concentration). Platelet vWf:Ag measurements were performed within 1 month of blood collection.

The multimeric composition of vWf was analyzed by sodium dodecyl sulphate (SDS) agarose gel electrophoresis, using low- or high-resolution gels and a discontinuous buffer system according to the method of Ruggeri and Zimmerman [19]. Low-resolution gels which better resolve large vWf multimers and were obtained with 1.2% low-gelling temperature agarose; high-resolution gels which better resolve the smaller multimers were prepared using 2.2% high-gelling temperature agarose. After electrophoresis, the gels were covered with rabbit ^{125}I-anti-human vWf purified antibody (Dako, Glostrup, Denmark). Autoradiographs were analyzed by a densitometer scanner (LKB, Upsala, Sweden).

RESULTS

Table II reports the main haemostatic findings in our HSP patients during active disease. In most cases PRP appeared to aggregate at ristocetin doses lower than in normal subjects (0.71 mg/ml ± 0.1 vs. normal 1.2 mg/ml ± 0.2), but the degree of platelet aggregation obtained with 1.5 mg/ml was normal. Plasma factor VIII:C and vWf levels were significantly higher in three of the patients studied (Table II). In all the others, the values appeared to be within the normal range. No relationship was found between vWf levels and the agglutinating re-

TABLE II. Haemostatic Findings for HSP Patients Evaluated in Active Disease

Patients	RIPA[a] %	MADR[b] mg/ml	VIII:C %	vWf:Ag %	vWf:RCoF %	Plat.vWf:Ag %	Platelets $\times 10^9$/L
1	68	0.75	100	91.8	72.5	86.2	265
2	83.4	0.75	102	47.5	50	62.5	275
3	—	—	67	65	63	—	519
4	—	—	162.5	107.4	112.5	—	455
5	69	0.75	84.0	93.7	59.3	—	320
6	70	0.60	204	218.2	224	142.5	288
7	77.9	0.75	116	192.5	138	—	252
8	83	0.75	153	239.4	213	175	275
Normal range	58–82	1.0–1.5	60–160	60–160	60–130	60–150	170–450

[a]Ristocetin-induced platelet aggregation.
[b]Minimal aggregating doses ristocetin.

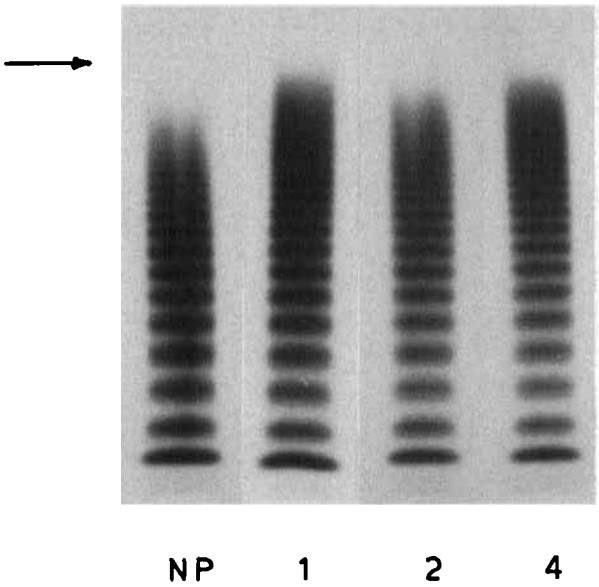

Fig. 1. Multimeric pattern of plasma vWf in patients with HSP. Electrophoresis was performed in 1.2% low-gelling temperature agarose. Multimers were detected by ^{125}I-labelled purified anti-human vWf antibody, followed by autoradiography. The origin of the running gel is at the top (arrow). From left to right: normal plasma (NP), plasma from patients 1, 2, and 4. It can be seen that, despite supranormal vWf multimers, each oligomer appears to be as well represented as the normal counterpart.

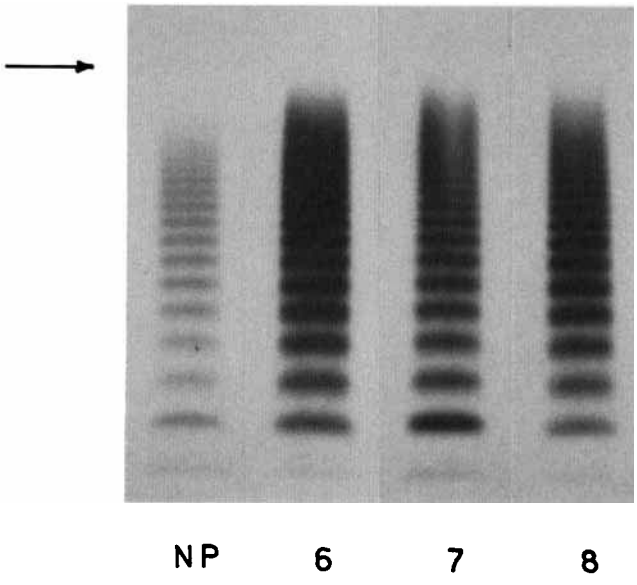

Fig. 2. Multimer pattern in the patients with HSP showing increased factor VIII:C and vWf levels. From left to right: normal plasma (NP) and plasma from patients 6, 7, and 8. Conditions as described in the legend to Figure 1.

sponse to ristocetin since increased platelet responsiveness was presented in each instance. Platelet vWf:Ag contents were measured in four of the patients, and were found to be normal or slightly increased.

Patient plasma was analyzed during active disease by SDS-agarose gel electrophoresis with a low resolution power (1.2%) which permits partial resolution of high molecular weight vWf multimers; we observed a set of larger multimers which were not present in normal plasma. This abnormality was documented both in patients with normal plasma vWf levels (Fig. 1) and in patients with increased values (Fig. 2).

In patients with increased plasma vWf levels, each oligomer, regardless of its molecular weight, appeared augmented but with electrophoretic mobility similar to that of patients with normal vWf levels. In both groups of patients, the proportion of abnormally large multimers was similar, suggesting that their presence is independent from vWf levels. No difference was observed when antiproteases were added to the anticoagulant (data not shown). No relationship between the number of the acute episodes and the larger vWf multimers representation could be demonstrated.

The patients also were studied 3 and 9 months later, during remission. On these occasions, no significant changes in the levels of vWf:Ag, vWf:RCoF, and factor VIII:C were observed in the patients with normal levels at admission. Conversely, in the others, a progressive

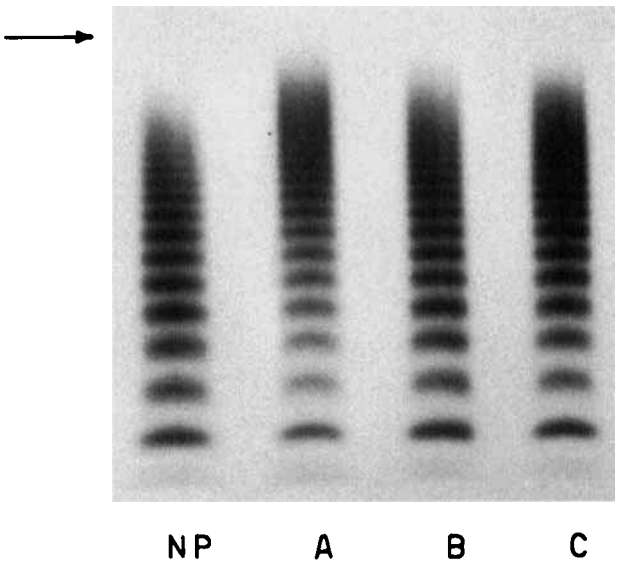

Fig. 3. vWf multimer analysis by SDS 1.2% agarose gel electrophoresis obtained from normal (NP) and patient 1 samples, collected during the active disease (A) and 3 and 9 months later (B,C), and during the remitting period. For more details see the legend to Figure 1.

decrease up to the normal levels at 9 months was observed. No significant differences in the platelet vWf content and in the increased agglutinating platelet response to ristocetin was found. On the contrary, larger vWf multimers were still significantly represented even though slightly decreased (Fig. 3).

DISCUSSION

This study demonstrates the existence of an altered plasma vWf multimeric structure in HSP patients, consisting of the presence of supranormal multimers similar to those normally found in cellular compartments, i.e., in platelet alfa-granules and in endothelial cell Weibel-Palade bodies [20]. Endothelial cell injury or intense stimulation promotes the secretion of unusually large vWf multimers from Weibel-Palade bodies [21,22]. They are processed as soon as after secretion [23] so that they are not usually present in normal plasma, with the exception of fetal and neonatal plasma due to a late maturation of endothelial cells during neonatal life [24].

Pharmacological conditions, such as drug administration [25], autoimmune vasculitis, or connective tissue diseases [26] have been associated with vascular injury and the appearance of vWf multimers larger than those normally present in circulation.

The vWf molecule has a heterogeneous size because of its polymeric nature, and is composed of identical subunits held together by disulfide bonds. Small multimers are secreted constitutively by endothelial cells, while the largest forms are released only after stimulation, through what is called the regulated pathway of secretion [4]. The largest vWf multimers are the most effective in haemostasis, particularly in the interaction between platelets and subendothelium [27].

The detection of supranormal vWf multimers in HSP vasculitis suggests a perturbation in and/or damage to endothelial cell function [28] possibly due to circulating IgA-containing immune complexes, which are most likely involved in the etiology of the disease. In our patients, we observed, both increased and normal/decreased levels of vWf, but in each case we found supranormal multimers. Therefore, the agent(s) responsible for endothelial cell perturbation either induces an excessive release of larger vWf multimers, or their reduced storage in Weibel-Palade bodies, or their decreased clearance from the circulation, rather than increased synthesis.

In addition, vWf multimers do not seem to derive from platelets which also contain them due to the finding of normal or slightly increased levels in platelets.

Intriguingly, supranormal vWf multimers are still present in circulation when the disease is inactive suggesting that the mechanism(s) responsible for the abnormal circulating vWf forms is operative throughout the entire clinical course of the disease.

Furthermore, there is no relationship between the duration of the disease, the number of purpuric episodes, or the intensity of renal involvement and the degree of the vWf abnormality, thereby raising the question about the appearance and significance of supranormal vWf multimers. In any event, their persistence during the inactive phase may imply an endothelial abnormality in patients prone to develop immunocomplex-induced lesions and HSP.

It might well be that, upon a genetic predisposition leading to endothelial release of abnormal vWf multimers, a further insult promoted by IgA-containing immune complexes, exposure to xenoagents, infectious bacteria, and drugs could reach a critical point turning into the appearance of purpuric episodes.

Leaving aside a possible pathogenetic role for vWf multimers in HSP, it appears that the demonstration of an abnormal vWf multimeric pattern in blood might discriminate a population risk of developing HSP.

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REFERENCES

1. Lee HS, Koh HI, Kim MJ, Rha HY: Henoch-Schoenlein nephrits in adults: a clinical and morphology study. Clin Nephr 26:125–130, 1986.
2. Hunder GG, Arend WP, Bolch DA, Calabrese LH, Fauci AS, Fries JF, et al: The American College of Rheumatology 1990 criteria for the classification of vasculitis. Arthritis Rheum 33:1065–1144, 1990.
3. Handin RI, Wagner DD: Molecular and cellular biology of von Willebrand factor. In BS Coller (ed): "Progress in Haemostasis and Thrombosis." Philadelphia/Toronto: Saunders, 1989, pp 233–260.
4. Sporn LA, Marder VJ, Wagner DD: Inducible secretion of large biologically potent von Willebrand factor multimers. Cell 46:185–190, 1986.
5. Chopek MW, Girma JP, Fujikawa K, Davie EW, Titani K: Human von Willebrand factor: a multivalent protein composed of identical subunits. Biochem 25:3146–3155, 1986.
6. Kuprinski JM, Miller JL: Multimeric analysis of von Willebrand factor in megakaryocytes. Thromb Res 38:603–610, 1985.
7. Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD: Involvement of large plasma von Willebrand factor (vWf) multimers and unusually large vWf forms derived from endothelial cells in shear stress induced platelet aggregation. J Clin Invest 78:1456–1461, 1986.
8. Gralnick HR, Williams SB, Morisato DK: Effect of multimeric structure of the factor VIII/von Willebrand factor protein on binding to platelets. Blood 58:387–397, 1981.
9. Mannucci PM, Ruggeri ZM, Pareti FI, Capitanio A: DDAVP: a new pharmacological approach to the management of haemophilia A and von Willebrand disease. Lancet 1:869–872, 1977.
10. Casonato A, Sartori MT, Pontara E, Bertomoro D, Dannhauser D, Girolami A: Discrepant increase in factor VIII:C and von Willebrand factor after DDAVP infusion in a patient with variant von Willebrand's disease. Blood Coag Fibrinol 2:567–573, 1991.
11. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azocar J, et al: Unusually large plasma factor VIII:von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. N Engl J Med 307:1432–1435, 1982.
12. Mannucci PM, Lombardi R, Lattuada A, Ruggenenti P, Viganò GL, Barbui T, Remuzzi G: Enhanced proteolysis of plasma von Willebrand factor in thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome. Blood 74:978–983, 1989.
13. Ruggenenti P, Galbusera M, Cornejo RP, Bellavita P, Remuzzi G: Thrombotic thrombocytopenic purpura: evidence that infusion rather than removal of plasma induces remission of the disease. Am J Kidney Dis 21:314–318, 1993.
14. Bertaglia G, Belmonte P, Nordio M, Vianello A, Meneghel G, Ossi E, Borsatti A: Factor VIII related antigen multimeric pattern in IgA nephropathy (IgAN). In Michalopoulos CD (ed): "Proceedings of the Xth International Congress on Thrombosis, May 22–27, 1988, Athens, Greece," p. 150.
15. Mills A, Beat A, Bloch AD, Calabrese LH, Hunder GG, Arend WP, et al: The American College of Rheumatology 1990 criteria for the classification of Henoch-Schonlein purpura. Arthritis Rheum 33:14–21, 1990.
16. Gherardi R, Belec L, Mhiri C, Gray F, Lescs MC, Guillevin L, Wechsler J: The spectrum of vasculitis in human immunodeficiency virus-infected patients. Arthritis Rheum 36:1164–1174, 1993.
17. Casonato A, Fabris F, Vicariotto M, Girolami A: The evaluation of factor VIII antigen by a means of a simple slide test. Am J Clin Path 84:107–111, 1985.
18. Casonato A, Sartori MT, Pontara E, Zucchetto A, Dannhauser D, Patrassi G, Girolami A: Impaired release of tissue plasminogen activator (t-PA) following DDAV infusion in von Willebrand's disease with low platelet von Willebrand factor content. Blood Coag Fibrinol 3:149–153, 1992.
19. Ruggeri ZM, Zimmerman TS: Variant von Willebrand disease: characterization of two subtypes by analysis of multimeric composition of factor VIII/von Willebrand factor in plasma and platelet. J Clin Invest 65:1318–1325, 1980.
20. Weibel ER, Palade GE: New cytoplasmic components in arterial endothelial. J Cell Biol 23:101–112, 1964.
21. Levine JD, Harlan JM, Harker LA, Joseph ML, Counts RB: Thrombin-mediated release of factor VIII antigen from human umbilical vein endothelial cells in culture. Blood 60:531–534, 1982.
22. Ribes JA, Francis CW, Wagner DD: Fibrin induces release of von Willebrand factor from endothelial cells. J Clin Invest 79:117–123, 1987.
23. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colamino NM, Azocar J, et al: Unusually large factor VIII:von Willebrand factor multimers in chronic relapsing thrombocytopenic purpura. N Engl J Med 307: 1432–1442, 1982.
24. Weinstein M, Blonchard R, Moake J, Vosburg E, Moise K: Fetal and neonatal von Willebrand factor (vWf) is unusually large and similar to the vWf in patients with thrombotic thrombocytopenic purpura. Brit J Haematol 72:68–72, 1989.
25. Charba D, Moake JL, Harris MA, Hester JP: Abnormalities of von Willebrand factor multimers in drug-induced-associated thrombotic microangiopathies. Am J Haematol 42:268–277, 1993.
26. Nusinow SB, Federici AB, Zimmerman TS, Gurd JC: Increased von Willebrand factor in the plasma of patients with vasculitis. Arthritis Rheum 27:1405–1410, 1984.
27. Federici AB, Bader R, Pagani S, Colibretti ML, De Marco L, Mannucci PM: Binding of von Willebrand factor (vWf) to glycoproteins (GP) Ib and IIb-IIIa complex: affinity is related to multimeric size. Br J Haematol 73:93–99, 1989.
28. Pearson JD: Markers of endothelial perturbation and damage. Br J Rheumatol 32:651–652, 1993.